BRIEFER ARTICLES.

THE ORGANS OF ATTACHMENT IN BOTRYTIS VULGARIS.

(WITH PLATE XVII)

WHILE engaged during the present year with a series of cultures of the common greenhouse fungus familiarly known as *Botrytis vulgaris*, the attention of the writer was attracted by the facility with which conidiophores and organs of attachment mutually replace each other as the result of changes of external conditions. The interest of such facts from a theoretical standpoint makes it seem worth while to record the following observations which were conducted under the direction of Professor V. M. Spalding.

Preliminary artificial cultures were prepared, each in a drop of malt solution on a glass slide. The first two, although as nearly alike as possible, showed very different results. One in the course of three days was completely covered with conidiophores (*fig. 1*), while in the other these were almost entirely absent, a development of peculiar cellular masses, the so-called organs of attachment, having taken place instead (*fig. 6*). The phenomenon suggested at once a relation of some kind between the organs of attachment and the conidiophores.

Other cultures were then prepared on slides, both plane and with concave centers, in test tubes, flasks, Petri dishes, etc., with the result that whenever the hyphæ came in contact with the hard surface of the culture dishes, these peculiar bodies appeared, and whenever the hyphæ grew upward without contact, conidiophores developed.

In order to determine with certainty whether or not the formation of conidiophores and organs of attachment could be controlled at will, the following cultures were prepared: In a drop of malt solution, placed on each of several slides with concave centers, were sown one or more conidia, and over the drop was suspended, by means of strips of damp blotting paper, a coverslip (2×1^{in}). These cultures were then transferred to moist chambers until wanted. The slips were placed at various heights, if close to the drop ($1-2^{mm}$ above) in two or three days the entire overhanging surface was covered by organs of attachment, while

conidiophores were formed only where hyphæ escaped at the sides and grew freely upward. When the slips were placed at greater heights (3–7^{mm}), a greater development of conidiophores followed with a corresponding decrease of organs of attachment.

Next slips of mica were perforated with the point of a fine needle and placed at various heights as in the above cultures, with the result that organs of attachment were formed where hyphæ came in contact with the overhanging surface, and conidiophores where they grew opposite or through the perforations. The fungus seemed to be entirely indifferent to light and gravity, and the formation of organs of attachment to be conditioned solely by the contact of hyphæ with a hard surface.

The development of the conidiophores and organs of attachment throws still farther light on their relation to each other. For the study of the developing conidiophores rather old perianths of *Lilium candidum* were inoculated with conidia from a clean culture and placed in moist chambers. In the course of three or four days the entire substratum was covered by the fleecy mycelium. From time to time this was examined until all the stages of development were obtained. Briefly stated they are as follows: Strong erect hyphæ, rich in protoplasm, branch two or three times, and from these branches repeated secondary divisions are sent out (*fig. 2*). Ultimately the apices of the branches swell, and peg like protuberances (*fig. 2 a*) appear which rapidly increase in size and number so that in a comparatively short time mature ovoid conidia (*fig. 2 b*) are developed from them. This formation of conidia was in progress at 3 o'clock P.M. April 14. Thus, with respect to the time of spore formation, this fungus differs from the one described by Klein.[1]

For the study of the successive stages of development of the organs of attachment, the following agar-agar cultures were prepared: malt solution, 2 per cent.; malt solution, 3 per cent.; geranium decoction, 2 per cent.; potato decoction, 2 per cent.; grape sugar, 2 per cent. In all of these media the fungus grew luxuriantly, producing organs of attachment when contact was possible, and conidiophores when this was impossible. The actual development of the organs of attachment was traced from a single conidium sown in 2 per cent. potato agar medium, as follows:

[1] Ueber die Ursachen der ausschliesslich-nächtlichen Sporenbildung von Botrytis cinerea. *Bot. Zeit.* 43: 6. 1885.

After germination of the conidium, the fungus grew rapidly, branching in every direction, and whenever an upright hypha came in contact with the cover of the shallow Petri dish (5.5^{mm} inside depth) repeated branching took place as in case of the conidiophores. As the organs increased in size the branches became shorter so that ultimately they were of no larger size than ordinary conidia of this species. The successive stages in the development are represented in *figs. 3–6*. *Fig. 3* shows the condition of things at 3:15 P. M.; *fig. 4* at 4:15 P. M.; *fig. 5* at 5:07; and *fig. 6* at 6 P. M. of the same day. After this the branching became so rapid and the organ so complex that accurate drawings were out of the question.

When we compare the successive stages in the development of the conidiophores and organs of attachment, we find them essentially alike in origin and mode of branching, but ultimately in the case of the developing conidiophore the tips swell and peg like projections are sent out which grow and become mature conidia, while in case of the organ of attachment the branching continues irregularly and indefinitely. Farther, when conidia are sown in nutrient solutions germ tubes are sent out, and when a nutrient solution is supplied to one of the organs of attachment each ultimate division sends out a tube resembling a germ tube. In both cases these hyphæ branch and develop into a complex mycelial mass. The resemblance therefore is a physiological as well as a morphological one.

The connection between conidiophores and organs of attachment is farther emphasized by the intermediate forms found in almost every culture. *Fig. 7*, drawn accurately, as the others are, with the camera lucida, represents a prostrate branch with a young organ of attachment at *b*, and short, erect branches bearing conidia at *a*. It was a common occurrence for a hypha to bear conidia in successive clusters until it came in contact with the cover of the Petri dish, when it produced an organ of attachment.

Up to this time the organs of attachment were studied under artificial conditions, but later attention was directed toward their formation and significance in nature. Leaves in connection with vigorously growing plants were tied in such a manner as to be from 2–4^{mm} above each other, conidia having been sown previously, either in a small drop of water or malt solution, on the under leaf. The plants were then placed in an atmosphere saturated with moisture, under a large bell jar. In no case was penetration observed where conidia were sown in

a drop of water simply, but where they were started in nutritive media, whether malt solution or a very small dead insect, in two or three days the fungus had penetrated both leaves, the one on which the conidia were sown as well as the one from 2–4^{mm} above it. Organs of attachment formed freely on the overhanging surface here as in the artificial cultures already described, while on the lower surface they were rarely met with. Whenever organs of attachment had thus formed tubes were sent out from them into the host in the manner described by former writers. But that the fungus is not confined to this means of entering the host was seen in sections of a leaf on which conidia had been sown in a drop of malt solution four days previous to sectioning. Here contact with other leaves was impossible, so that the erect hyphæ bore conidia, while the prostrate ones ran over the surface of the leaf until a stoma was found for entrance (*fig. 8*). Again, some of the conidia produced by this mycelium had germinated and sent germ-tubes directly into the leaf between the lamellæ of two adjacent cells (*fig. 9*). In the latter case the tissue of the leaf was probably weakened by the presence of the fungus in some other part, since after entrance the tissue is rapidly consumed.

From the foregoing experiments, and others not recorded, the writer concludes that with this fungus the formation of organs of attachment is determined by external conditions which may be artificially produced by placing in proximity to the hyphæ a hard surface for contact, or they may be met with in nature where plants are crowded together so that the leaves of different plants lap over each other; that when this condition is not present conidiophores will be developed instead of organs of attachment; that in general conidiophores and organs of attachment are both physiologically and morphologically equivalent; that biologically the fungus makes use of the organs of attachment to penetrate a neighboring leaf, or it has the alternative, after starting saprophytically, of entering the host either through the stomata or by sending germ tubes directly into the tissue.

The literature of *Botrytis vulgaris* and allied forms is considerable, yet comparatively little has been said concerning the morphology of the organs of attachment. Brefeld[2] classes them, on account of their manner of attaching themselves to a substratum, with other "Haft organen," while De Bary[3] throws more light on their biological rela-

[2] Schimmelpilze 4; 112.

[3] Bot. Zeit. 44: 382, 412. 1886.

HORN on BOTRYTIS.